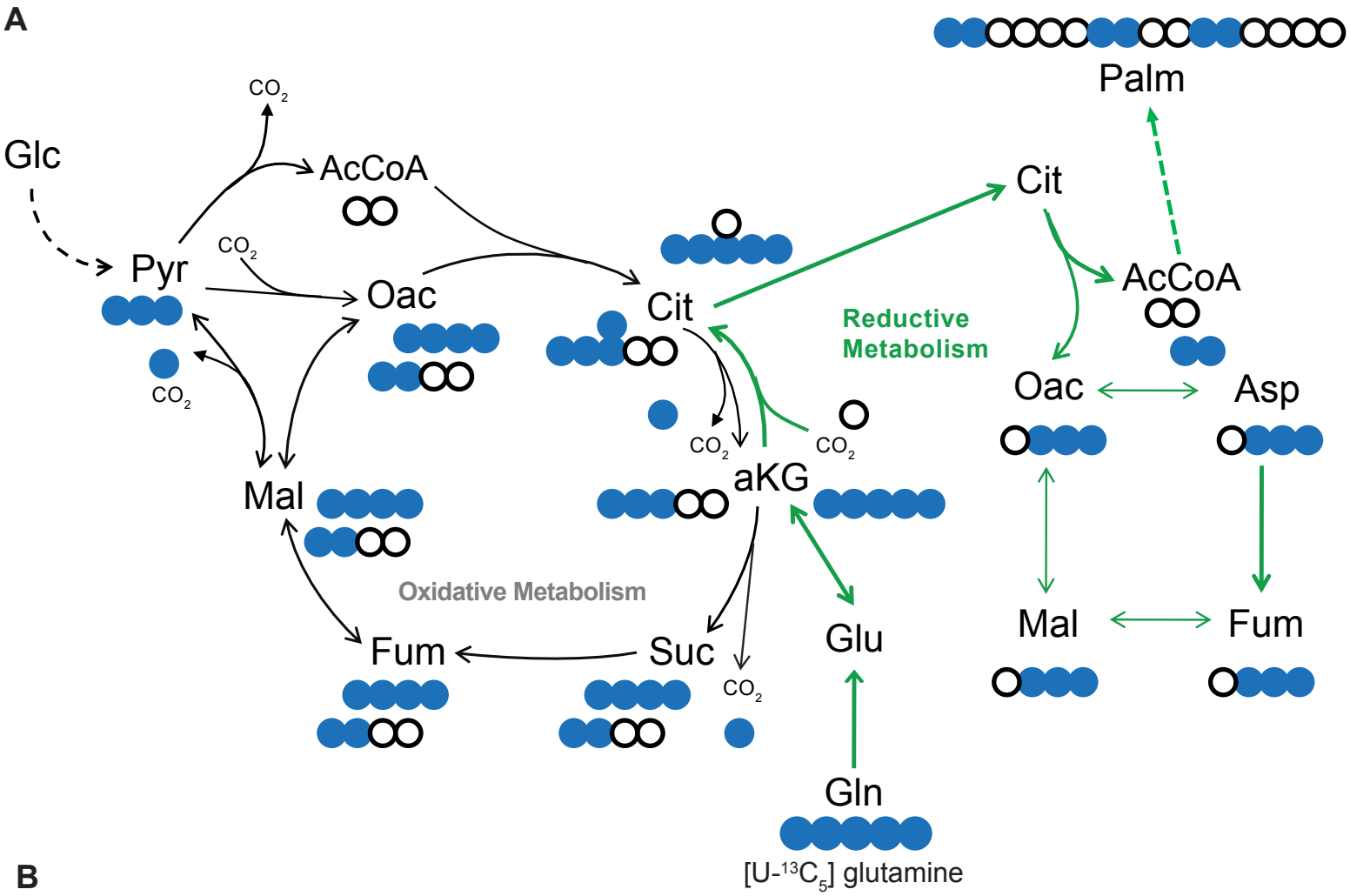
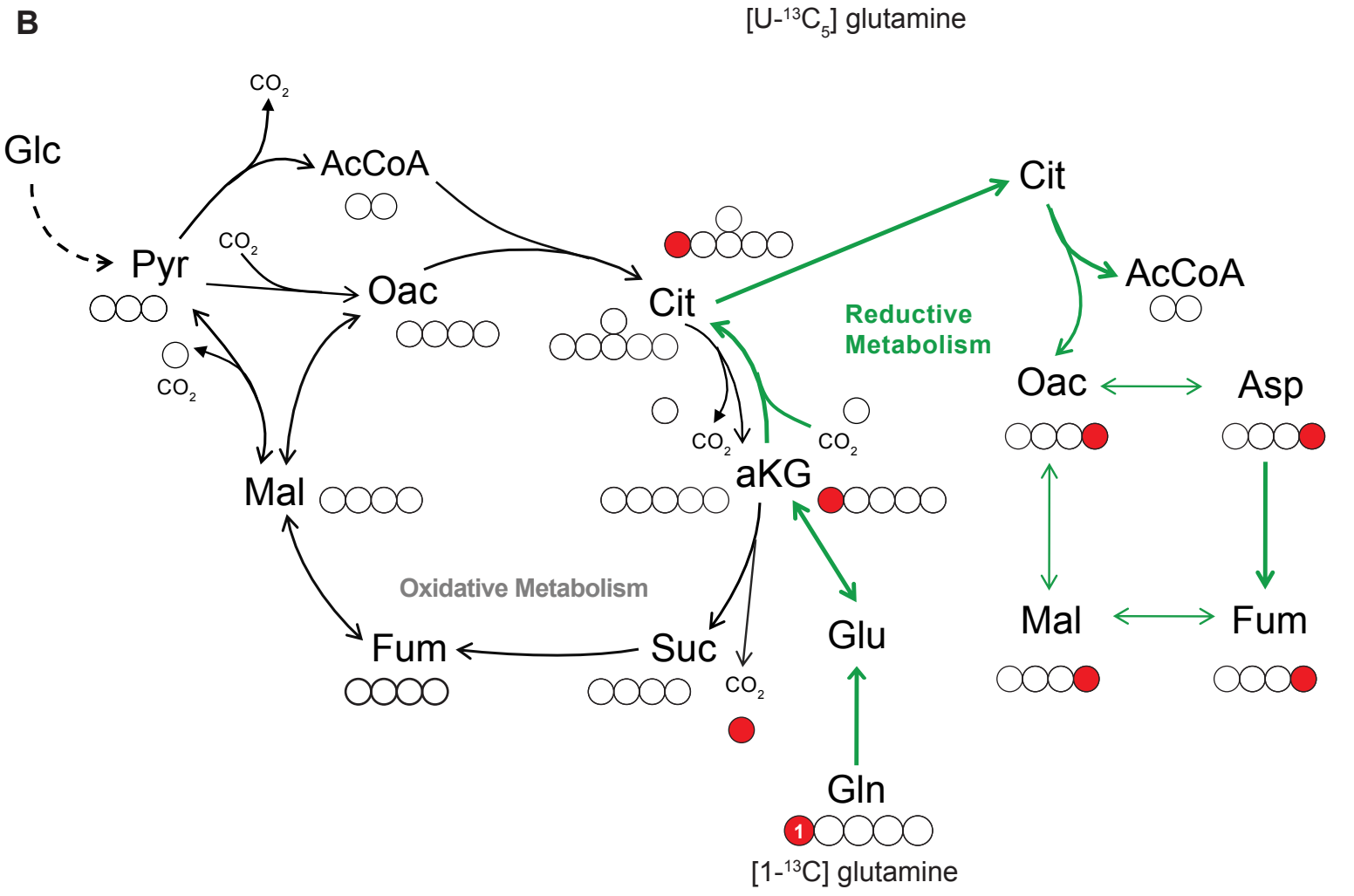
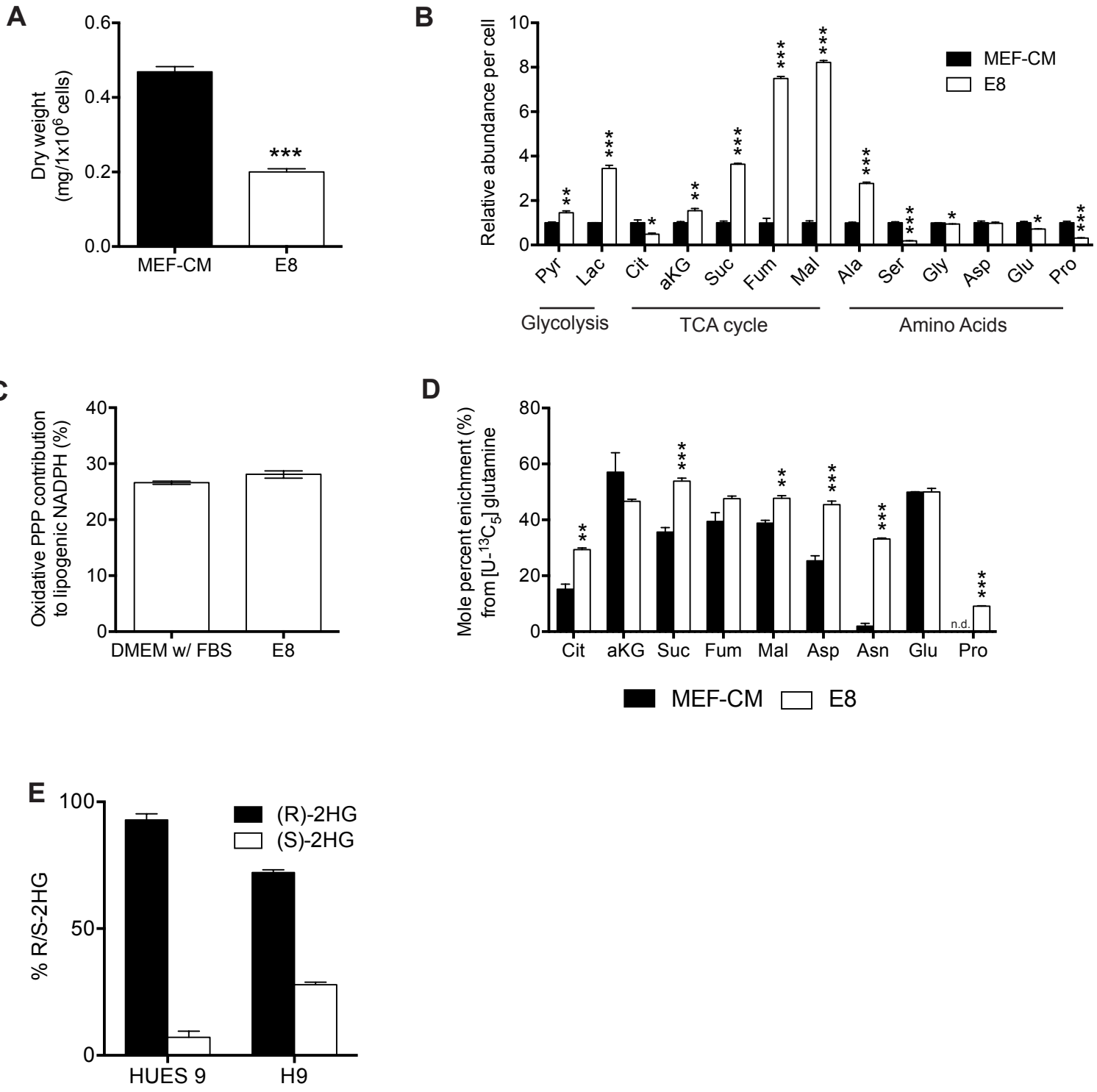
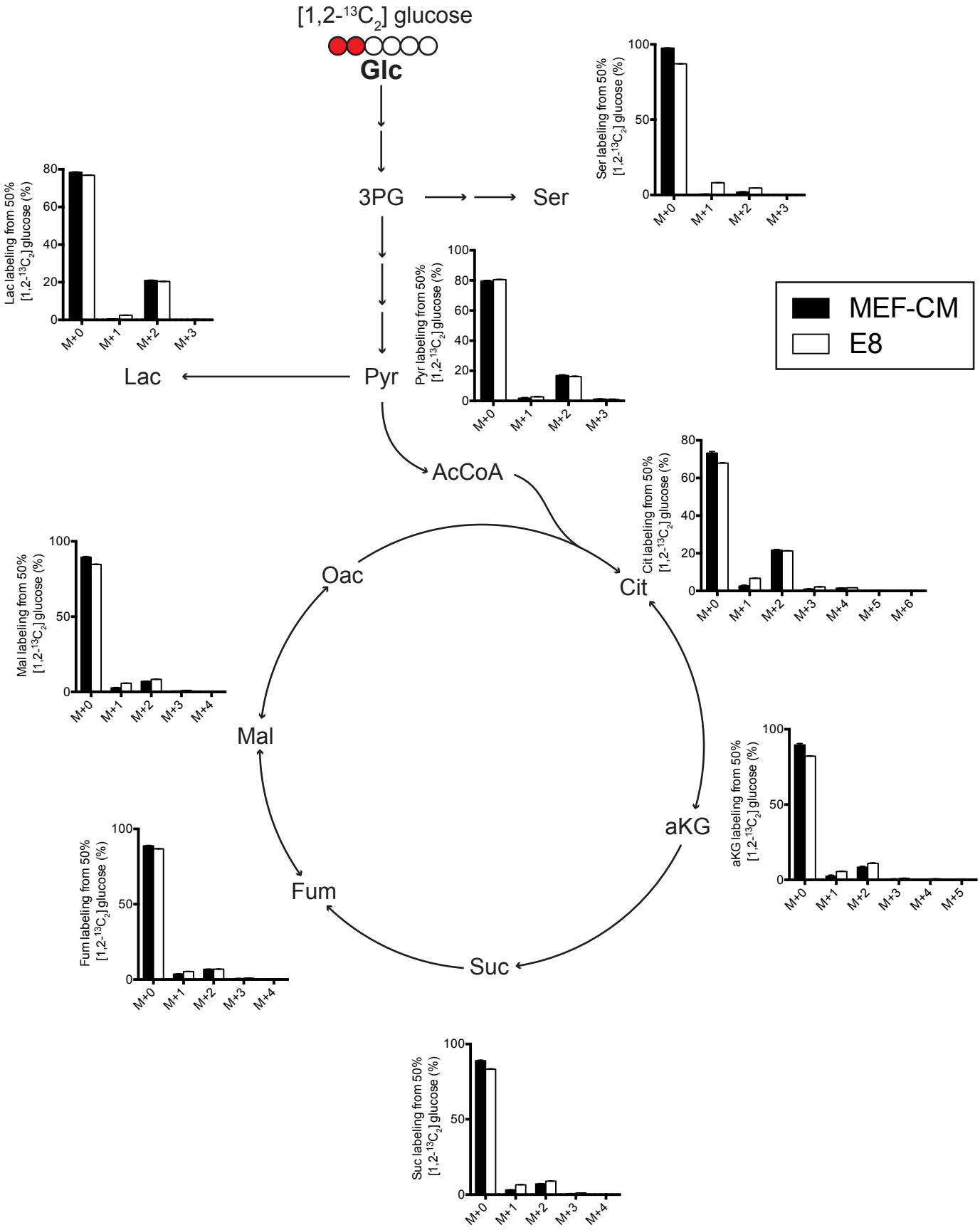
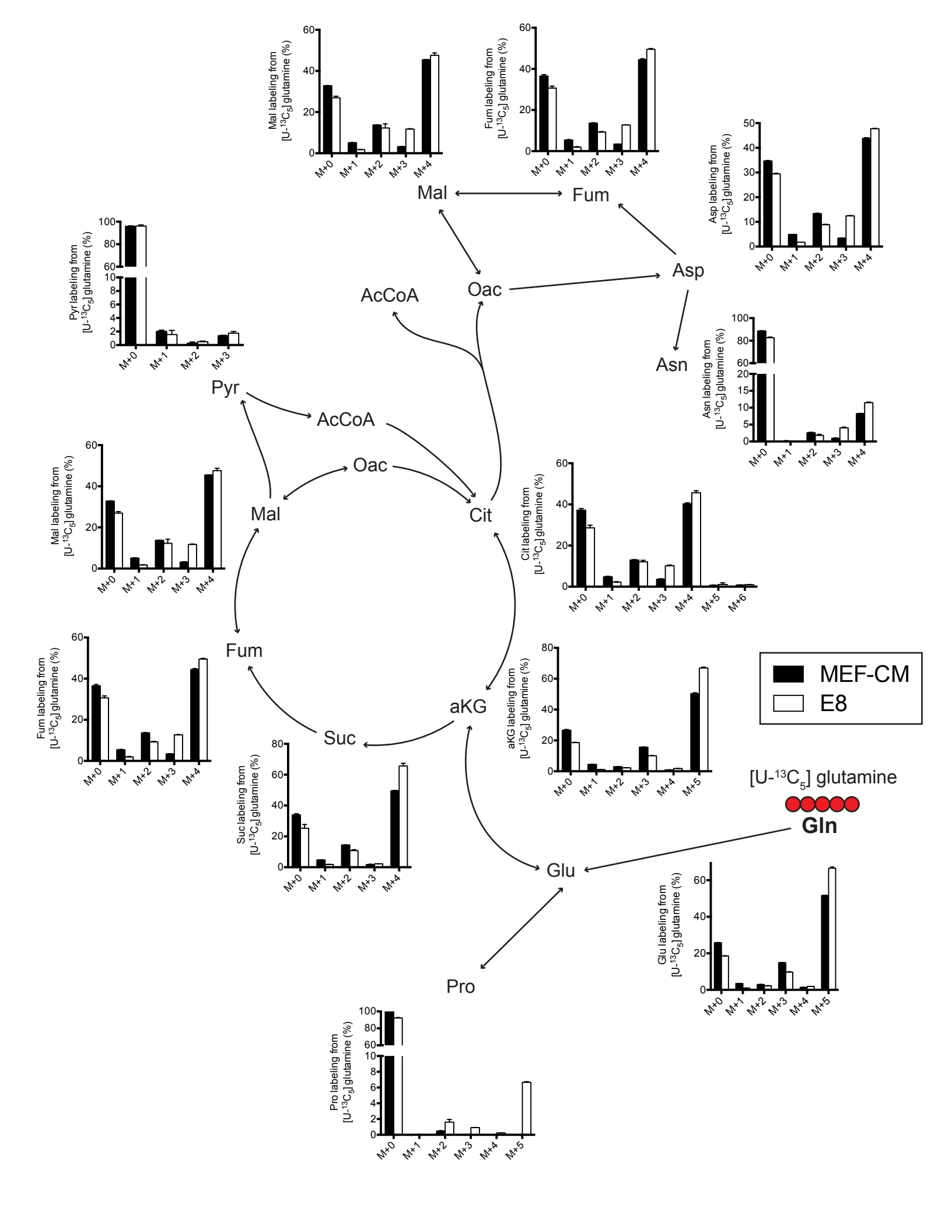
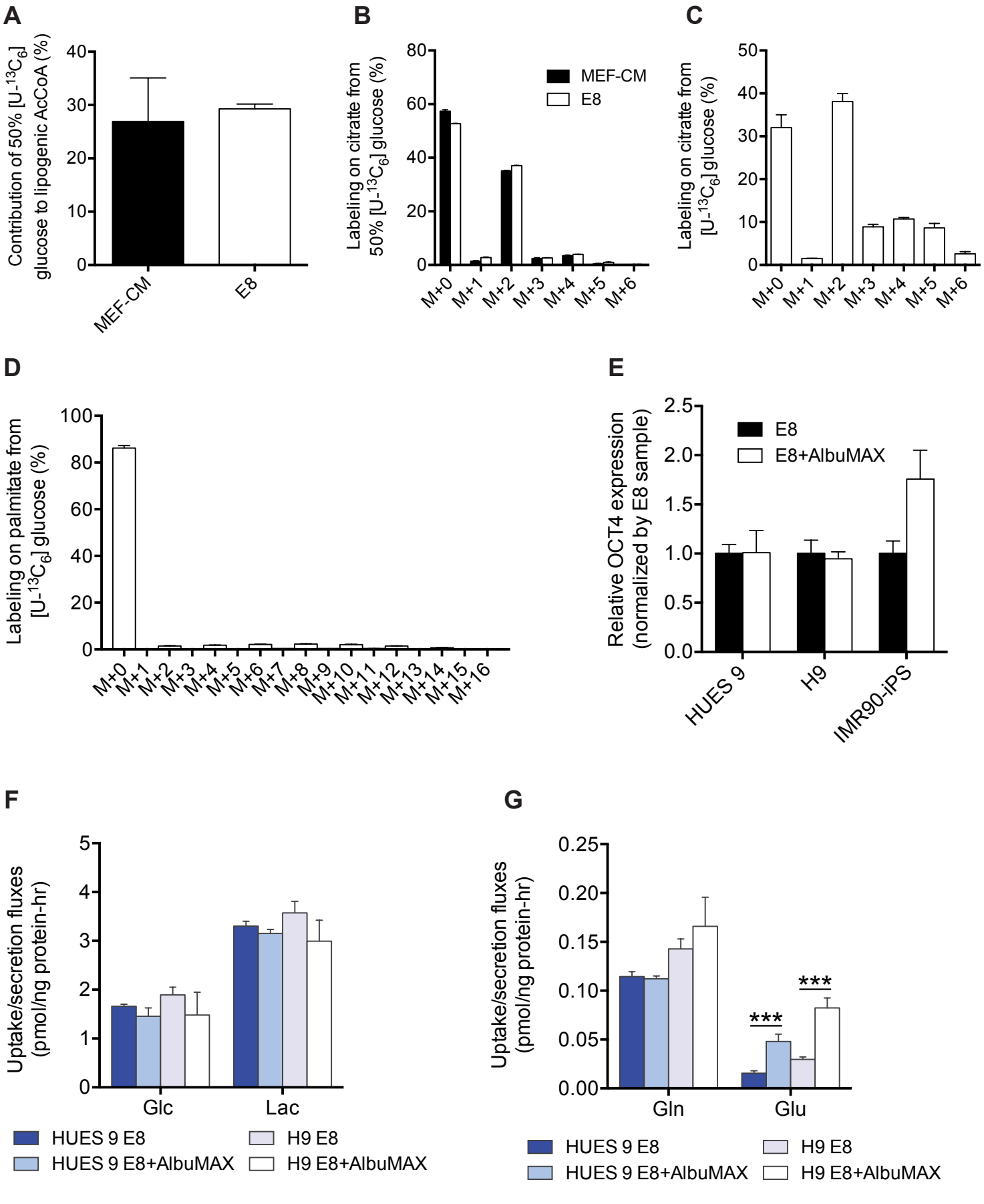

A
Palm
Glc
CO2
AcCoA
Cit
Pyr
CO2
Oac
Cit
AcCoA
Reductive
Metabolism
CO2
Oac
Asp
CO2
CO2
aKG
Mal
Oxidative Metabolism
Glu
Fum
Suc
Mal
Fum
CO2
Gln
[U-13C5] glutamine

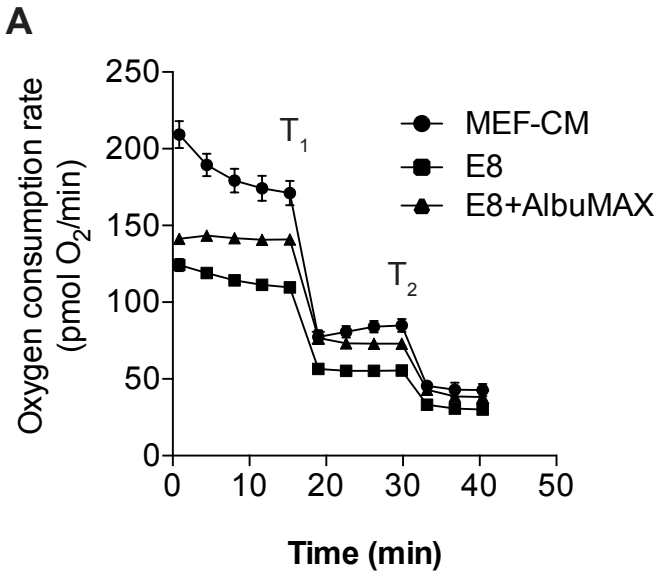

B
Glc
CO2
AcCoA
Cit
Pyr
CO2
Oac
Cit
AcCoA
Reductive
Metabolism
CO2
Oac
Asp
CO2
CO2
aKG
Mal
Oxidative Metabolism
Glu
Fum
Suc
Mal
Fum
CO2
Gln
1
[1-13C] glutamine

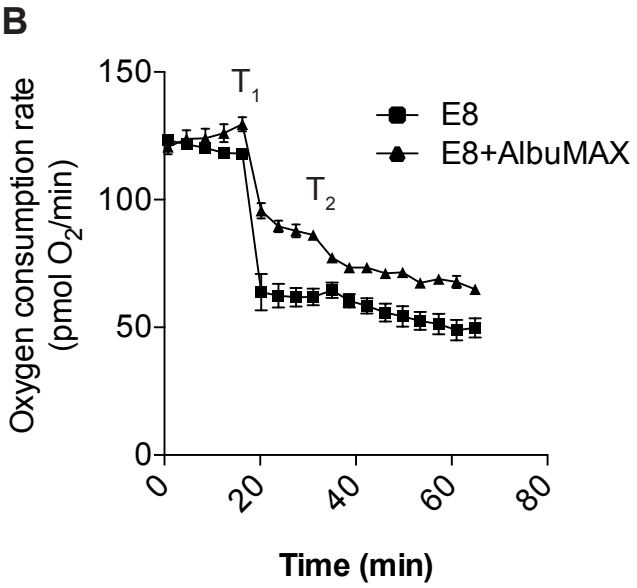

A
Dry weight (mg/1x10^6 cells)
0.6
0.4
0.2
0.0
***
MEF-CM
E8
B
Relative abundance per cell
10
8
6
4
2
0
MEF-CM
E8
Pyr
Lac
Cit
aKG
Suc
Fum
Mal
Ala
Ser
Gly
Asp
Glu
Pro
Glycolysis
TCA cycle
Amino Acids
C
Oxidative PPP contribution to lipogenic NADPH (%)
40
30
20
10
0
DMEM w/ FBS
E8
D
Mole percent enichment (%) from [U-13C5] glutamine
80
60
40
20
0
Cit
aKG
Suc
Fum
Mal
Asp
Asn
Glu
Pro
n.d.
MEF-CM
E8
E
% R/S-2HG
100
50
0
(R)-2HG
(S)-2HG
HUES 9
H9

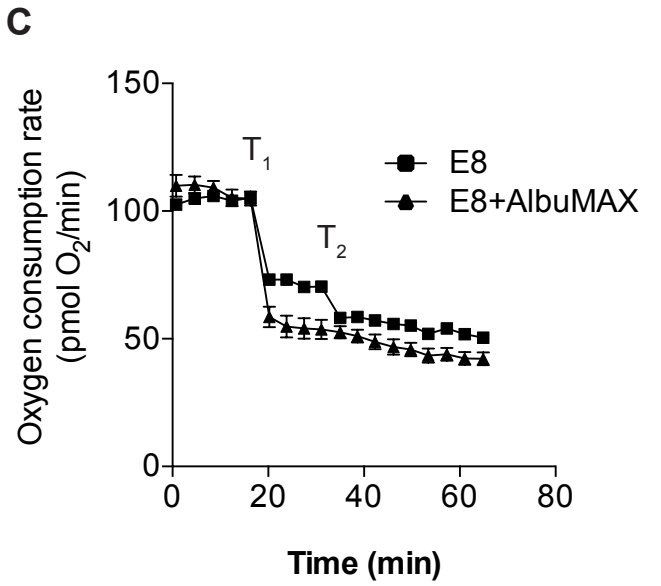

[1,2-13C2] glucose
Glc
3PG
Ser
Lac
Pyr
AcCoA
Cit
aKG
Suc
Fum
Mal
Oac
MEF-CM
E8
Ser labeling from 50% [1,2-13C2] glucose (%)
Lac labeling from 50% [1,2-13C2] glucose (%)
Pyr labeling from 50% [1,2-13C2] glucose (%)
Cit labeling from 50% [1,2-13C2] glucose (%)
Mal labeling from 50% [1,2-13C2] glucose (%)
aKG labeling from 50% [1,2-13C2] glucose (%)
Fum labeling from 50% [1,2-13C2] glucose (%)
Suc labeling from 50% [1,2-13C2] glucose (%)
M+0
M+1
M+2
M+3
M+4
M+5
M+6


Mal labeling from [U-13C5] glutamine (%)
Fum labeling from [U-13C5] glutamine (%)
Asp labeling from [U-13C5] glutamine (%)
Pyr labeling from [U-13C5] glutamine (%)
Asn labeling from [U-13C5] glutamine (%)
Mal labeling from [U-13C5] glutamine (%)
Cit labeling from [U-13C5] glutamine (%)
Fum labeling from [U-13C5] glutamine (%)
aKG labeling from [U-13C5] glutamine (%)
Suc labeling from [U-13C5] glutamine (%)
Glu labeling from [U-13C5] glutamine (%)
Pro labeling from [U-13C5] glutamine (%)
M+0
M+1
M+2
M+3
M+4
M+5
M+6
Mal
Fum
Asp
Oac
AcCoA
Asn
Pyr
AcCoA
Oac
Mal
Cit
Fum
aKG
Suc
Glu
Pro
MEF-CM
E8
[U-13C5] glutamine
Gln


A
Contribution of 50% [U-13C6] glucose to lipogenic AcCoA (%)
40
30
20
10
0
MEF-CM
E8
B
Labeling on citratte from 50% [U-13C6] glucose (%)
80
60
40
20
0
MEF-CM
E8
M+0
M+1
M+2
M+3
M+4
M+5
M+6
C
Labeling on citratte from [U-13C6] glucose (%)
50
40
30
20
10
0
M+0
M+1
M+2
M+3
M+4
M+5
M+6
D
Labeling on palmitate from [U-13C6] glucose (%)
100
80
60
40
20
0
M+0
M+1
M+2
M+3
M+4
M+5
M+6
M+7
M+8
M+9
M+10
M+11
M+12
M+13
M+14
M+15
M+16
E
Relative OCT4 expression (normalized by E8 sample)
2.5
2.0
1.5
1.0
0.5
0.0
E8
E8+AlbuMAX
HUES 9
H9
IMR90-iPS
F
Uptake/secretion fluxes (pmol/ng protein-hr)
5
4
3
2
1
0
Glc
Lac
HUES 9 E8
HUES 9 E8+AlbuMAX
H9 E8
H9 E8+AlbuMAX
G
Uptake/secretion fluxes (pmol/ng protein-hr)
0.25
0.20
0.15
0.10
0.05
0.00
***
***
Gln
Glu
HUES 9 E8
HUES 9 E8+AlbuMAX
H9 E8
H9 E8+AlbuMAX


A
Oxygen consumption rate (pmol O2/min)
250
200
150
100
50
0
0
10
20
30
40
50
T1
T2
MEF-CM
E8
E8+AlbuMAX
Time (min)


B
Oxygen consumption rate (pmol O2/min)
150
100
50
0
0
20
40
60
80
T1
T2
E8
E8+AlbuMAX
Time (min)


C
Oxygen consumption rate (pmol O2/min)
150
100
50
0
0
20
40
60
80
T1
T2
E8
E8+AlbuMAX
Time (min)

Supplemental Figure Legends

Figure S1, Related to Figure 2. Atom transition maps of labeled glutamine species. Metabolite abbreviations described in Supplemental Text. (A) Schematic of atom transitions in the presence of [U-$^{13}C_5$]glutamine. ^{12}C carbons depicted with open circles. ^{13}C carbons depicted with filled circles. Dashed lines indicate multi-step atom transitions. M+(n) indicates the number (n) of ^{13}C atoms incorporated into the metabolite. M+5 citrate and M+3 oxaloacetate, aspartate, fumarate, and malate indicative of reductive glutamine flux. M+3 a-ketoglutarate and M+2 succinate, fumurate, and malate indicative of oxidative glutaminolysis. (B) Schematic of atom transitions in the presence of [1-^{13}C]glutamine. Labeled carbon lost in oxidative TCA flux. M+1 labeling indicative of reductive TCA flux.

Figure S2, Related to Figure 1 and 2. Metabolic alterations in hESCs adapted to MEF-CM versus chemically defined media. HESCs were adapted to MEF-CM and chemically defined media for at least 3 passages. (A) Dry cell weight per million H9 hESCs. (B) Relative intracellular metabolite abundance of H9 hESCs normalized by cell number and MEF-CM sample. (C) Percentage of oxidative PPP contribution to lipogenic NADPH in A549 cells cultured in DMEM with 10% FBS or E8 as determined by ISA using [3-^{2}H]glucose. (D) Mole percent enrichment from [U-$^{13}C_5$]glutamine in H9 hESCs throughout intermediary metabolism. (E) Relative percentage of 2-HG isoforms in hESCs grown in E8. (A-B, D-E) All results shown as mean ± SEM. P values were calculated using a Student's two-tailed *t* test relative to MEF-CM condition; *, P value between 0.01 and 0.05; **, P value between 0.001 and 0.01; ***, P value <0.001. (C) Results shown as mean and 95% CI. *, Significance indicated by non-overlapping 95% confidence intervals.

Figure S3, Related to Figure 2. Mass isotopomer distributions from [1,2-$^{13}C_2$]glucose. HUES 9 cells were adapted to MEF-CM and chemically defined media for at least 3 passages. Steady state mass isotopomer distributions (labeling) of metabolites throughout central carbon metabolism in cells cultured with a 1:1 mixture of unlabeled glucose and [1,2-$^{13}C_2$]glucose over 24 hours. All results shown as mean ± SEM. M+(n) indicates the number (n) of ^{13}C atoms incorporated into the metabolite. Metabolite abbreviations described in Supplemental Text.

Figure S4, Related to Figure 2. Mass isotopomer distributions from [U-$^{13}C_5$]glutamine. HUES 9 cells were adapted to MEF-CM and chemically defined media for at least 3 passages. Steady state mass isotopomer distributions (labeling) of TCA metabolites and amino acids from [U-$^{13}C_5$]glutamine after 24 hours. All results shown as mean ± SEM. M+(n) indicates the number (n) of ^{13}C atoms incorporated into the metabolite. Metabolite abbreviations described in Supplemental Text.

Figure S5, Related to Figure 4. Nutrient lipids in MEF-CM impacts hESC metabolism. (A) Glucose contribution to lipogenic AcCoA in HUES 9 hESCs in the presence 50% enriched [U-$^{13}C_6$]glucose. (B) Mass isotopomer distribution (labeling) of citrate in HUES 9 hESCs in the presence of 50% enriched [U-^{13}C]glucose over 24 hours. (C) Mass isotopomer distribution of citrate in irradiated CF-1 MEFs in the presence of 50% enriched [U-$^{13}C_6$]glucose after 24 hours of media conditioning. (D) Mass isotopomer distribution of palmitate in irradiated CF-1 MEFs in the presence of 50% enriched [U-$^{13}C_6$]glucose over 24 hours. (E) Expression of OCT4 in hPSCs adapted to E8+AlbuMAX relative to cells in E8. (F-G) Glucose uptake, lactate secretion, glutamine uptake and glutamate secretion fluxes of hESCs adapted to E8 or E8+AlbuMAX. Cells were adapted to E8 and E8+AlbuMAX for at least 3 passages. (A) Results shown as mean with 95% CI. *, significance determined by non-overlapping confidence intervals. (B-G) All results shown as mean ± SEM. P values were calculated using a Student's two-tailed *t* test relative to MEF-CM condition; *, P value between 0.01 and 0.05; **, P value between 0.001 and 0.01; ***, P value <0.001.

Figure S6, Related to Figure 5. Oxygen consumption traces of hPSCs in different culture conditions. (A) Representative traces of HUES 9 hESC oxygen consumption rate (OCR). Oligomycin is

added at time T_1 and rotenone/antimycin A is added at time T_2. (B) Representative traces of H9 hESC oxygen consumption rate (OCR). Oligomycin is added at time T_1 and rotenone/antimycin A is added at time T_2. (C) Representative traces of IMR90-iPS hPSC oxygen consumption rate (OCR). Oligomycin is added at time T_1 and rotenone/antimycin A is added at time T_2. (A-C) All results shown as mean ± SEM.

Metabolite Abbreviations

AcCoA, acetyl-CoA; **αKG**, α-ketoglutarate; **Ala**, alanine; **Asp**, aspartate; **Asn**, asparagine; **Lac**, lactate; **Cit**, citrate; **Fum**, fumarate; **Glc**, glucose; **Glu**, glutamate; **Gln**, glutamine; **Gly**, glycine; **2HG**, 2-hydroxyglutarate; **Mal**, malate; **Oac**, oxaloacetate; **Olea**, oleate; **Palm**, palmitate; **3PG**, 3-phosphoglyceric acid; **Pro**, proline; **Pyr**, pyruvate; **Ru5P**, ribulose-5-phosphate; **Ser**, serine; **Suc**, succinate.

Supplemental Procedure

Cell culture and media

Human embryonic stem cell lines HUES9 and WA09 (H9) were provided by Prof. Shyni Varghese (University of California, San Diego) and Prof. Sean Palecek (University of Wisconsin-Madison), respectively. HESCs were originally maintained on a layer of irradiated CF-1 murine embryonic fibroblasts (P3, MEFs) (MTI-GlobalStem) in DMEM/F12 medium with 20% knockout serum replacement (KSR), 1X MEM non-essential amino acid solution (NEAA), 1 mM L-glutamine, 1X 2-mercaptoethanol (2-ME), and 4 ng/ml basic fibroblast growth factor recombinant human protein (bFGF). All components were purchased from Life Technologies. Induced pluripotent stem cell line iPS(IMR90)-c4 was also provided by Prof. Sean Palecek. IPSCs were originally cultured in mTeSR1 medium (Stem Cell Technologies). All hPSCs experiments were conducted with cells ranging from 30 and 70 passages.

MEF-conditioned medium was produced by culturing 1 million P3 irradiated CF-1 MEF (MTI-GlobalStem) in 10ml DMEM/F12 medium with 20% KSR, 1X NEAA, 1 mM L-Glutamine and 1X 2-ME The conditioned medium was collected every 24 hours from day 2 to day 7 and pooled. Before culturing hESC, the conditioned medium was supplemented with fresh 10 ng/ml bFGF. All components were purchased from Life Technologies.

AlbuMAX media was made by dissolving AlbuMAX I Lipid-Rich BSA (Life Technologies; 1-1.6% w/v) and ultra-fatty acid free BSA (Roche; 1% w/v) into E8 basal media or tracer E8 basal media. E8 supplement was then freshly added to lipid-containing basal media.

In [U-$^{13}C_{16}$] palmitate tracer experiments, [U-$^{13}C_{16}$] palmitate was first non-covalently conjugated to ultra-fatty acid free BSA (Roche) by dissolving [U-$^{13}C_{16}$]sodium palmitate (Cambridge Isotopes) to a concentration of 2.5 mM in 150 mM sodium chloride solution at 70°C and adding 40 ml palmitate solution into 50 ml of 0.34 mM BSA solution at 37°C. A 1 mM working BSA-conjugated [U-$^{13}C_{16}$] palmitate solution was prepared by adjusting the pH to 7.4 and diluting to a final volume of 100 ml with 150 mM sodium chloride. In experiments, 50 μM BSA-conjugated [U-$^{13}C_{16}$] palmitate and 1 mM carnitine were added to culture medium.

Human cancer cell lines, H1299, HCT116, 143B, SW1353, H358, Hep3b, Huh7 and A549, were maintained in DMEM supplemented with 10% fetal bovine serum (FBS). For measurement of oxidative PPP contribution to lipogenic NADPH, tracer media consisted of either glucose free DMEM medium with 10% dialyzed FBS or glucose free E8 medium, supplemented with [3-^{2}H] glucose (Cambridge Isotopes). All components were purchased from Life Technologies.

For tracer experiments, culture medium was removed, cells were rinsed with PBS, and tracer media were added to wells. Cells were maintained in tracer media for 24 hours before metabolite extraction.

All cells were maintained in a humidified, 37°C incubator at 5% CO_2.

Detection of 2-hydroxyglutarate isoforms

Derivatization of 2-hydroxyglutarate (2HG) with methanol/methyl chloroformate (Sigma-Aldrich) was performed following essentially the protocol described previously (Villas-Boas et al., 2003). The derivatives were extracted by the addition of 70 μl of chloroform. To check the enantiomer separation and to evaluate retention times, standard solutions of both R-α-hydroxyglutaric acid disodium salt and S-α-hydroxyglutaric acid disodium salt (Sigma-Aldrich) were prepared and derivatized in the same way.

A sample volume of 2 μl was injected into a split/splitless inlet, operating in pulsed splitless mode

at 230 °C. The injection pulse pressure was set to 15 psi until 1 minute. The gas chromatograph was equipped with a Rt-γDEXsa (length: 30 m, I.D.: 0.25 mm, film: 0.25 µm) capillary column (Restek). The GC oven temperature was held at 70 °C for 1 minute and increased at 4 °C/min to 150 °C. After 5 minutes, the temperature was increased at 3 °C/min to 190 °C, then held at that temperature for 5 minutes. The total run time for each sample was about 40 minutes (Waldhier et al., 2010). The transfer line temperature was set constantly to 280 °C. Full-scan mass spectra were acquired from *m/z* 70 to 500. Other conditions are same as for other metabolite detection. For quantification, measurements of the derivatives were performed in SIM mode using the following masses: *m/z* 159, *m/z* 175.1 (quantification ion) and *m/z* 202.1. The dwell time for each ion was set to 150 ms. All GC-MS chromatograms were processed using MetaboliteDetector (Hiller et al., 2009).

References

Hiller, K., Hangebrauk, J., Jager, C., Spura, J., Schreiber, K. & Schomburg, D. (2009). MetaboliteDetector: comprehensive analysis tool for targeted and nontargeted GC/MS based metabolome analysis. Anal Chem*, 81***,** 3429-39.

Villas-Boas, S. G., Delicado, D. G., Akesson, M. & Nielsen, J. (2003). Simultaneous analysis of amino and nonamino organic acids as methyl chloroformate derivatives using gas chromatography-mass spectrometry. Anal Biochem*, 322***,** 134-8.

Waldhier, M. C., Dettmer, K., Gruber, M. A. & Oefner, P. J. (2010). Comparison of derivatization and chromatographic methods for GC-MS analysis of amino acid enantiomers in physiological samples. J Chromatogr B Analyt Technol Biomed Life Sci*, 878***,** 1103-12.

Table S1, related to Figures 1-4. Metabolite fragments used for GC/MS analysis.

Metabolite	Carbons	Derivatization	m/z	Fragments for integration
α-Ketoglutarate	1,2,3,4,5	tBDMS	346	$C_{14}H_{28}O_5NSi_2$
Alanine	1,2,3	tBDMS	260	$C_{11}H_{26}O_2NSi_2$
Aspartate	1,2,3,4	tBDMS	418	$C_{18}H_{40}O_4NSi_3$
Lactate	1,2,3	tBDMS	261	$C_{11}H_{25}O_3Si_2$
	2,3		233	$C_{10}H_{25}O_2Si_2$
Citrate	1,2,3,4,5,6	tBDMS	459	$C_{20}H_{39}O_6Si_3$
Fumarate	1,2,3,4	tBDMS	287	$C_{12}H_{23}O_4Si_2$
Glutamate	1,2,3,4,5	tBDMS	432	$C_{19}H_{42}O_4NSi_3$
Glycine	1,2	tBDMS	246	$C_{10}H_{24}O_2NSi_2$
2-Hydroxyglutarate	1,2,3,4,5	tBDMS	433	$C_{19}H_{41}O_5Si_3$
Malate	1,2,3,4	tBDMS	419	$C_{18}H_{39}O_5Si_3$
Norvaline	1,2,3,4,5	tBDMS	288	$C_{13}H_{30}O_2NSi_2$
Proline	1,2,3,4,5	tBDMS	330	$C_{16}H_{36}O_2NSi_2$
Pyruvate	1,2,3	tBDMS	174	$C_6H_{12}O_3NSi$
Serine	1,2,3	tBDMS	390	$C_{17}H_{40}O_3NSi_3$
Succinate	1,2,3,4	tBDMS	289	$C_{12}H_{25}O_4Si_2$
Cholesterol	1-27	TMS	458	$C_{30}H_{54}OSi$
Coprostan-3-ol	1-27	TMS	370	$C_{27}H_{45}$
Heptadecanoate	1-17	FAME	284	$C_{18}H_{36}O_2$
Oleate	1-18	FAME	296	$C_{19}H_{36}O_2$
Palmitate	1-16	FAME	270	$C_{17}H_{34}O_2$
Stearate	1-18	FAME	298	$C_{19}H_{38}O_2$

Table S2, related to Figures 3 and 5. Primers used for gene expression analysis.

Gene	Forward Primer	Reverse Primer	Primerbank ID
ACACA	TCACACCTGAAGACCTTAAAGCC	AGCCCACACTGCTTGTACTG	38679973c3
ACLY	ATCGGTTCAAGTATGCTCGGG	GACCAAGTTTTCCACGACGTT	38569422c2
FASN	AAGGACCTGTCTAGGTTTGATGC	TGGCTTCATAGGTGACTTCCA	41872630c1
GAPDH	CTGGGCTACACTGAGCACC	AAGTGGTCGTTGAGGGCAATG	378404907c3
G6PD	ACCGCATCGACCACTACCT	TGGGGCCGAAGATCCTGTT	108773794c2
GLS2	GGCCATGTGGATCGCATCTT	ACAGGTCTGGGTTTGACTTGG	20336213c3
OCT4	CTTGAATCCCGAATGGAAAGGG	CCTTCCCAAATAGAACCCCCA	4505967a3
SCD	TTCCTACCTGCAAGTTCTACACC	CCGAGCTTTGTAAGAGCGGT	53759150c3